

Flows in complex biochemical networks: Role of low degree nodes

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Abstract

Metabolic networks have two properties that are generally regarded as unrelated: One, they have metabolic reactions whose single knockout is lethal for the organism, and two, they have correlated sets of reactions forming functional modules. In this review we argue that both essentiality and modularity seem to arise as a consequence of the same structural property: the existence of low degree metabolites. This observation allows a prediction of (a) essential metabolic reactions which are potential drug targets in pathogenic microorganisms and (b) regulatory modules within biological networks, from purely structural information about the metabolic network.

Introduction

In the course of evolution organisms have developed redundancies in their intracellular networks so as to tolerate random failures such as the knockout of a single gene or reaction. Nevertheless certain genes or reactions are ‘essential’ for growth and their single knockout can render the cell unviable. A question arises: What makes a gene or reaction essential? Is there some special structural property characterizing essential reactions in metabolic networks? Here we review some of our work which identifies such a property and describes it in network terms. This work shows that most essential metabolic reactions in *E. coli*, *S. cerevisiae* and *S. aureus* can be explained by the fact that they are associated with a low degree metabolite [1].

A second property of biological networks is their modularity [2], which is important, among other things, for their robustness and evolvability. Modularity appears in several biochemical networks, including metabolic networks and genetic regulatory networks. One can ask: Are there structures at a lower level of biological organization such as metabolism, perhaps reflecting basic chemical constraints, that cause, or at least encourage, modularity to emerge at a higher and evolutionarily more flexible level of organization such as the genetic regulatory network? It seems that metabolites with a low degree of connectivity could provide one such structure. Low degree metabolites participate in very few reactions in the network, which may be due to some feature of their chemical structure that prohibits ready association with other molecules. By virtue of their low degree these metabolites also contribute to a rigidity or coherence of reaction fluxes in the network resulting in clusters of highly correlated reactions. It turns out that these reaction clusters correspond to genetic regulatory modules with high probability, as captured in the structure of operons in *E. coli* [1].

Thus we argue that low degree metabolites are implicated in two distinct properties of biochemical networks, one, the essentiality of certain metabolic reactions, and two, the modularity of genome organization.

Relationship between essential reactions and low degree metabolites

Essential metabolic reactions: Definition and in-silico identification

A metabolic reaction is designated as ‘essential’ for an organism in a given environmental condition if its single knockout from the network renders the organism unviable in that condition. Essential reactions are therefore critical for the survival of the organism; as such their very existence represents fragilities associated with metabolism. The experimental determination of all essential genes and associated reactions in an organism under different environmental conditions is extremely tedious and time consuming. Alternatively, in-silico methods such as flux balance analysis (FBA) can be used for fast prediction of all essential metabolic reactions in an organism under different environmental conditions.

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FBA is a computational modelling technique which can be used to obtain the optimal growth rate of the organism and the steady state fluxes of all reactions in the metabolic network for any given growth medium (for a review see ref. [3]). Using FBA, a reaction is said to be ‘essential’ for a particular medium, if switching the reaction off results in a zero optimal growth rate for that medium. In [1], FBA was used to compute essential reactions in the *E. coli* metabolic network (version iJR904 [4]) under 89 different aerobic minimal media. These 89 media correspond to all possible distinct single organic sources of carbon under which *E. coli* can grow in aerobic conditions, as determined using FBA. It was found that the number of essential reactions varied between 200 and 240 across the 89 minimal media. A reaction was designated as ‘globally essential’ for *E. coli*, if it was essential for all the 89 media. The number of globally essential reactions was obtained to be 164 for the *E. coli* metabolic network. We now turn to an explanation, in terms of the underlying structure of the *E. coli* metabolic network, as to why these reactions are essential.

Uniquely produced and uniquely consumed metabolites

The metabolic network may be represented as a bipartite graph consisting of two types of nodes: metabolites and reactions. In a directed bipartite graph, there are two types of links: (a) from metabolite nodes to reaction nodes and (b) from reaction nodes to metabolite nodes. A metabolite is designated as ‘uniquely produced’ or ‘UP’ (‘uniquely consumed’ or ‘UC’), if there is only a single reaction in the metabolic network that produces (consumes) the metabolite [1]. A UP(UC) metabolite has in-degree (out-degree) equal to unity in the bipartite graph. A metabolite that is both UP and UC is designated as a ‘UP-UC metabolite’. In general, a metabolite that is either UP or UC or both has a low degree in the metabolic network as it participates in very few reactions. Similarly, a reaction is designated as ‘uniquely producing’ or ‘UP’ (‘uniquely consuming’ or ‘UC’), if it produces (consumes) a UP(UC) metabolite in the bipartite metabolic network. Reactions in the metabolic network that are either UP or UC or both are designated as ‘UP/UC reactions’. Examples of UP(UC) metabolites and reactions in the *E. coli* metabolic network are shown in Fig. 1.

Almost all essential reactions are UP/UC

The *E. coli* metabolic network database iJR904 which contains 761 metabolites participating in 931 reactions was studied. Starting from the list of 931 reactions in the *E. coli* network, a transformed network containing 1176 reactions was constructed by replacing each reversible reaction with two irreversible reactions (one each for the forward and backward direction). In the reconstructed metabolic network databases discussed here, there are certain reactions that can only have a zero flux value under any steady state. Such reactions have been referred to as ‘blocked’ reactions [5, 6], and their occurrence points towards the incompleteness of metabolic network databases. 290 blocked reactions were found in the *E. coli* metabolic network; these were removed from the transformed network of 1176 reactions to obtain a reduced network of 886 reactions representing *E. coli* metabolism. The removal of blocked reactions from the original network does not affect the allowed steady state fluxes through the rest of the network, and in particular does not alter the set of essential reactions obtained using FBA. The number of UP(UC) reactions in the reduced metabolic network of *E. coli* was found to be 245 (218). The number of reactions that were either UP or UC or both (the ‘UP/UC reactions’) in the reduced metabolic network of *E. coli* was 352.

It is evident that if a UP (or UC) metabolite is an essential intermediate in the production of a metabolite that is part of the biomass reaction contributing to the growth of the organism then the reaction responsible for the production (or consumption) of that UP (or UC) metabolite becomes essential for the growth of the organism. However, the converse is not obvious, i.e., there is no reason why all or most essential reactions should be UP or UC; there could, in principle, be other reasons why a reaction is essential. Of the 164 globally essential reactions in the *E. coli* metabolic network, 156 were either UP or UC in the reduced network. This explains why the subset of 156 reactions are globally essential in *E. coli*. By definition, there exists some metabolite (that is presumably required for generating biomass) whose production or consumption can only be performed by these reactions, thereby making them essential. Further, we found the probability of such a high overlap between the set of globally essential reactions and set of UP/UC reactions occurring by pure chance is very small (see the *p*-value in Table 1). In Fig. 1, we have shown 14 UP/UC reactions that are globally essential reactions in the *E. coli* metabolic network; they are required for the production of the biomass metabolite lipopolysaccharide (abbreviated as *lps*_{EC} in Fig. 1). We also determined globally essential reactions in the metabolic networks of two other organisms, *S. cerevisiae* (version iND750 [7]) and *S. aureus* (version iSB619 [8]), and again found

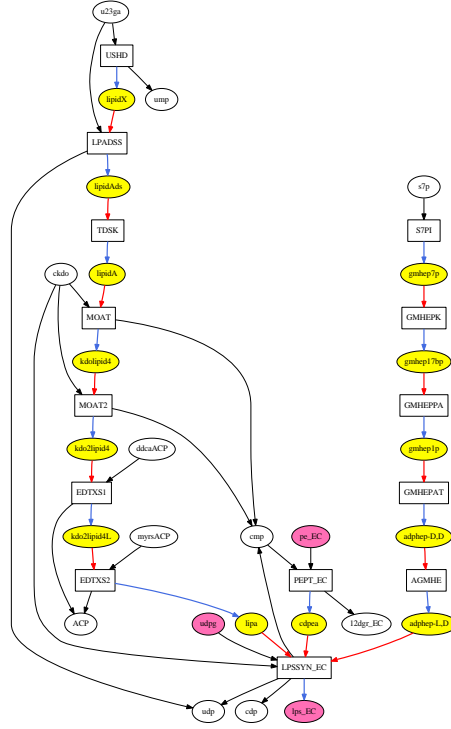


Figure 1: UP-UC metabolites in the *E. coli* metabolic network forming a UP-UC cluster. The 14 reactions that belong to the UP-UC cluster are ‘globally essential’. Rectangles represent reactions and ovals metabolites. Yellow ovals represent UP-UC metabolites and pink ovals represent biomass metabolites. A blue (red) link represents the production (consumption) of a UP (UC) metabolite; otherwise the link is shown in black. To reduce clutter, nodes corresponding to *adp*, *atp*, *h*, *h2o*, *pi* and *ppi* have been omitted. Abbreviation of metabolite and reaction names are as in [4].

almost all globally essential reactions in the two organisms to be either UP or UC in their respective reduced networks (see Table 1).

Mahadevan and Palsson [9] had earlier observed that the lethality fraction (the fraction of reactions a metabolite is involved in that are essential) of the low degree metabolites is on average comparable to high degree metabolites. However, they did not realize that the essential reactions are explained by their association with the low degree metabolites rather than the high degree ones. Our work has led to the understanding that the essential reactions may involve other metabolites of higher degree, but their essentiality is due to their uniqueness in producing or consuming a UP or UC metabolite [1].

It was also found that of the 352 UP/UC reactions in the *E. coli* reduced metabolic network 288 were essential in at least one of the 89 aerobic minimal media. The intersection of the so-called high-flux backbone reactions [10] across these media also consists primarily of UP/UC reactions (116 out of 124). There exist multiple flux vectors (or multiple solutions of FBA) with the same optimal growth rate in any given medium [11]. This multiplicity represents the redundancy in metabolic pathways. The high-flux backbone changes across the multiple solutions. The part of the high-flux backbone that is conserved across the multiple solutions also consists primarily of the UP/UC reactions (160 out of 197 in glucose minimal medium) [12]. These facts underline the importance of UP/UC reactions in maintaining flows across metabolic networks.

UP-UC clusters predict regulatory modules and are network motifs

A UP-UC metabolite has one reaction that produces it and one reaction that consumes it in the metabolic network. A steady state is defined as one where all metabolite concentrations and reaction velocities are constant. In any steady state, the flux of the reaction producing a UP-UC metabolite is always proportional to the flux of the reaction consuming the metabolite. A ‘UP-UC cluster’ of reactions was defined as a set of reactions connected

Organism	<i>E. coli</i>	<i>S. cerevisiae</i>	<i>S. aureus</i>
Number of reactions in the transformed network	1176	1579	865
Number of reactions in the reduced network	886	779	571
Number of globally essential reactions	164	127	196
Number of globally essential reactions that are UP or UC	156 ($p < 10^{-62}$)	117 ($p < 10^{-41}$)	182 ($p < 10^{-58}$)

Table 1: Comparison of globally essential reactions and UP/UC reactions in the metabolic networks of *E. coli*, *S. cerevisiae* and *S. aureus*. This table is adapted from Ref. [1]

by UP-UC metabolites [1]. An example of a UP-UC cluster of reactions in the reduced metabolic network of *E. coli* is shown in Fig. 1. In steady state, fluxes of all reactions that are part of a single UP-UC cluster are proportional to each other and fixing the flux of any reaction in a UP-UC cluster fixes the fluxes of all other reactions in the cluster under steady state. UP-UC clusters of reactions are special cases of reaction/enzyme subsets [13], co-sets [11] and fully coupled reactions [6] that have been discussed earlier in the literature. Further, for any steady state analysis, each UP-UC cluster can be replaced by a single effective reaction, and this can be used to coarse-grain metabolic networks [13].

Since the fluxes of reactions forming a UP-UC cluster have fixed ratios with respect to each other for all steady states, the set of genes that code for enzymes catalyzing various reactions of the cluster may be expected to be coregulated forming a transcriptional module. In prokaryotes genes are grouped into transcriptional modules called operons. The set of genes that form a single operon are always transcribed together. We have investigated whether the genes coding for enzymes catalyzing reactions of a UP-UC cluster are part of the same operon in *E. coli*. It was found [1] that from the set of 251 genes corresponding to reactions of all the UP-UC clusters, two genes belonging to the same UP-UC cluster have a much greater probability (more than 50 times) of lying on the same operon than a randomly chosen pair (the probabilities are 0.29 and 0.0057, respectively). Thus, the set of genes that correspond to a UP-UC cluster in the *E. coli* metabolic network are strongly correlated with regulatory modules at the genetic level.

The bunching up of UP-UC metabolites next to each other in the metabolic network results in the formation of UP-UC clusters with more than two reactions. A natural question arises: Is it expected for a network like the *E. coli* metabolic network to have many large UP-UC clusters? This question may be answered by comparing with a null model. The distribution of UP-UC clusters in the real *E. coli* metabolic network was compared with a suitably randomized version of the original network. It was found that the real metabolic network of *E. coli* has its UP-UC metabolites bunched up next to each other, forming larger clusters than may be expected in random networks with the same local connectivity properties as the original network [1]. ‘Network motifs’ have been defined as patterns of interconnections that occur in different parts of a network at frequencies much higher than those found in randomized networks [14]. Thus, larger size UP-UC clusters may be collectively considered as analogous to a network motif, since they are over-represented in the real *E. coli* metabolic network. The larger UP-UC clusters in the real network may facilitate the regulation of certain metabolic pathways inside the organism.

Discussion

Metabolic networks inside different organisms have been shown to follow a power law degree distribution [15, 16] which is characterized by the presence of high degree metabolites referred to as ‘hubs’. It has been suggested that one of the important consequences of a power law degree distribution is the vulnerability of the network to selective attack on hubs while being robust to random deletion of nodes from the network [17]. For the protein-protein interaction network of *S. cerevisiae*, it was shown that the essentiality of a protein is correlated with its degree in the network [18]. This observation has been suggested as evidence for the importance of the hubs in maintaining the overall structure and function of cellular networks. Although the role of high degree metabolites or hubs in maintaining the overall structure of the metabolic networks has been well emphasized in the literature, the role of low degree metabolites has attracted little or no attention. In our study, we find that certain low degree metabolites explain globally essential reactions and introduce fragility for flows in metabolic network. We have further shown that the low degree metabolites as opposed to high degree metabolites explain essential reactions in metabolic networks [1]. Thus, it is the low degree metabolites that are critical from the point of view of functional robustness of the metabolic system.

The fragility caused by low degree metabolites in metabolic networks can have potential applications in medicine. In our work, we have observed that essential reactions are explained by UP/UC structure in three organisms. Thus, it is likely that UP/UC structure explains essential reactions in other organisms that are pathogens for humans. This generates candidate targets for therapeutic intervention. It is conceivable that drugs could be found that incapacitate the enzymes catalyzing essential reactions in pathogens.

The above work highlights the essentiality of reactions associated with low degree metabolites. It also shows that these metabolites lead to correlated clusters of reactions in the metabolic network that preferentially correspond to genetic regulatory modules in *E. coli*. Thus the same structural property the existence of low degree metabolites seems to be associated with two seemingly unrelated properties of the system: essentiality and modularity. While the essentiality of a reaction is obviously connected with a fragility of the organism (knockout of the reaction is lethal), modularity is generally believed to contribute to the organism's robustness and evolvability. This might be an example of a rather general property of complex systems, that their robustness in some dimension often goes hand in hand with fragility in another dimension [19].

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